Thymidine synchronization of in vitro development of bovine embryos

UNIVERSITÀ DEGLI STUDI DI TORINO

THYMIDINE SYNCHRONIZATION OF *IN VITRO* DEVELOPMENT OF BOVINE EMBRYOS

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Excess thymidine is capable of synchronizing lymphocyte cultures *in vitro* by acting with a feed-back mechanism during the S phase of the cell cycle (Harper 2005 Methods Mol. Biol. 296, 157–166). The possibility to synchronize the embryonic growth can be a good strategy for future epigenetic studies. The present study was undertaken to test whether excess thymidine could also synchronize *in vitro* development of bovine embryos. Abattoir-derived cumulus–oocyte complexes (COC) of the Agerolese breed of cattle were matured *in vitro* using standard procedures. After maturation, COCs were transferred in drops of 300 µL of IVF-TALP (25/drop) and covered with mineral oil. Frozen sperm from a bull were selected by centrifugation on a Percoll discontinuous gradient (45 to 80%). The pellet was diluted in IVF medium and added to the COC at the concentration of 1×10^6 sperm mL^{-1}. After 18–20 h of gametes co-incubation, presumptive zygotes were denuded and cultured in SOF medium containing different concentrations of thymidine (0, 300, 600, 1200, 2400 µg mL^{-1}, final concentrations). The day after (Day 2) presumptive zygotes were washed four times in fresh SOF, classified morphologically under a stereomicroscope as not divided (n.d.), 2 cells, 3–8 cells, and 9–32 cells and cultured in standard SOF at 39°C in a humidified mixture of 5% CO_2,7% O_2, and 88% N_2. On Day 3, the embryos were again examined for the growth stage in relation to the synchronization effects. On Day 7, the embryos were evaluated for the final growth efficiency (cleavage stage and blastocyst formation). The experiment was replicated 4 times (except the 2400 µg mL^{-1} condition, which was replicated 2 times because of its clear toxic effect). Data were analyzed by ANOVA test. At Day 2, there were no differences between groups whatever the concentration (n.d.–2 cells: 59.7 ± 11.2, 52.9 ± 26.3, 56.1 ± 14.6, 66.0 ± 2.8, 38.0 ± 11.3; 3–8 cells : 40.3 ± 11.2, 47.1 ± 26.3, 43.9 ± 14.6, 34.0 ± 2.8, 59.9 ± 9.5; respectively for 300, 600, 1200, 2400, and control), while differences at Day 3 and 7 are shown in Table 1. The dosage of 300 µg mL^{-1} slowed embryo development without altering the developmental rate, whereas the other dosages were somewhat toxic to the zygotes, affecting the final percentage of blastocysts.

Table 1. State of development of zygotes on Day 3 and Day 7 (cleavage and embryo rate)

Thymidine (µg mL^{-1})	Zygotesanalyzed (No.)	Day 3, No. (% ± SD)			Day 7, No. (% ± SD)	
		n.d.[1]–2 cells	3–8 cells	9–32 cells	Cleaved	Bl
300	94	42 (44.5 ± 0.9)	44 (46.8 ± 1.5)^{A}	8 (8.6 ± 1.1)^{a}	63 (68.3 ± 7.3)	17 (19.6 ± 8.5)^{a}
600	120	54 (41.7 ± 18.8)	58 (50.4 ± 13.1)	8 (7.9 ± 7.1)^{a}	78 (67.6 ± 19.5)	14 (12.2 ± 4.9)
1200	117	56 (46.5 ± 14.4)	51 (45.7 ± 14.2)^{a}	10 (7.8 ± 5.6)	82 (70.2 ± 13.3)	7 (6.7 ± 5.8)^{Bb}
2400	50	23 (46.0 ± 2.8)	26 (52.0 ± 0.0)^{Bb}	1 (2.0 ± 2.8)^{B}	30 (15.0 ± 1.4)	0
Control	114	45 (35.8 ± 16.8)	45 (41.2 ± 7.8)^{A}	24 (23.0 ± 9.0)^{Ab}	82 (23.0 ± 9.0)	22 (21.2 ± 7.5)^{A}

^{A,B,C}Values within columns with different letters are different; $P < 0.01$; ^{a,b,c}values within columns with different letters are different; $P < 0.05$.
[1]Not divided.